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Dated -9 AUG 1994

For official use

27 NOV 1992

-1DEC'92#00285581 PAT 1 77 UC 25.00

27 NOV 1992

- 9224880.6

Your reference 135279/2

Notes £25

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The Patent Office

Request for grant of a Patent

Form 1/77

Patents Act 1977

❶ Title of invention

1 Please give the title of the invention STEROIDS

❷ Applicant's details

❑ **First or only applicant**

2a If you are applying as a corporate body please give:

Corporate name BRITISH TECHNOLOGY GROUP LTD

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2b If you are applying as an individual or one of a partnership please give in full:

Surname

Forenames

2c **In all cases**, please give the following details:

Address BRITISH TECHNOLOGY GROUP LTD
101 NEWINGTON CAUSEWAY
LONDON

UK postcode *(if applicable)* SE1 6BU

Country

ADP number *(if known)* 609552001

003

2d, 2e and 2f: *If there are further applicants please provide details on a separate sheet of paper.*

❑ **Second applicant** ***(if any)***

2d If you are applying as a corporate body please give:

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Agent's name MR. R. K. PERCY

Agent's address PATENTS DEPT
BRITISH TECHNOLOGY GROUP LTD
101 NEWINGTON CAUSEWAY
LONDON

Postcode SE1 6BU

Agent's ADP number 4083507004

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❹ Reference number

4 Agent's or applicant's reference number *(if applicable)* 135279/2

❺ Claiming an earlier application date

5 Are you claiming that this application be treated as having been filed on the date of filing of an earlier application?

Please mark correct box

Yes ☐ No ☒ ➡ ***go to 6***

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15(4) (Divisional) ☐ 8(3) ☐ 12(6) ☐ 37(4) ☐

❻ Declaration of priority

❻ *If you are declaring priority from a PCT Application please enter 'PCT' as the country and enter the country code (for example, GB) as part of the application number.*

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7 Are you (the applicant or applicants) the sole inventor or the joint inventors?

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Yes ☐ No ☒ ➡ ***A Statement of Inventorship on Patents Form 7/77 will need to be filed (see Rule 15).***

❽ *Please supply duplicates of claim(s), abstract, description and drawing(s).*

❽ Checklist

8a Please fill in the number of sheets for each of the following types of document contained in this application.

Continuation sheets for this Patents Form 1/77			—
Claim(s)	3	Description	31
Abstract	1	Drawing(s)	—

8b Which of the following documents also accompanies the application?

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Priority documents *(please state how many)*	
Translation(s) of Priority documents *(please state how many)*	
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Patents Form 9/77 – Preliminary Examination/Search	
Patents Form 10/77 – Request for Substantive Examination	

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Signed Keith Perry Date 27 November 1992
(day month year)

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135279/2

STEROIDS

Background of the invention

1. Field of the invention

This invention relates to steroids and their use in the treatment of androgen-dependent and oestrogen-dependent disorders, especially prostatic cancer and breast cancer respectively.

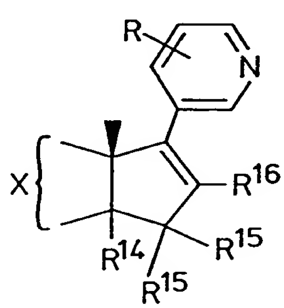
2. Description of the related art

The 17α-hydroxylase/C_{17-20} lyase enzyme complex (hereinafter "hydroxylase/lyase") is known to be essential for the biosynthesis of androgens and oestrogens. In the treatment of androgen-dependent disorders, especially prostatic cancer, there is a need for strong inhibitors of hydroxylase/lyase. Certain anti-androgenic steroids are well known, for example Cyproterone acetate (17α-acetoxy-6-chloro-1α,2α-methylene-4,6-pregnadiene-3,20-dione). Many other steroids have been tested as hydroxylase/lyase inhibitors. See, for example, PCT Specification WO 92/00992 (Schering AG) which describes anti-androgenic steroids having a pyrazole or triazole ring fused to the A ring at the 2,3- position, or European Specifications EP-A 288053 and EP-A 413270 (Merrell Dow) which propose 17β-cyclopropylamino-androst- 5-en-3β-ol or -4-en-3-one and their derivatives.

Summary of the invention

It has now surprisingly been found that steroids lacking a C_{20} side chain and having a 17-(3-pyridyl) group in its place, together with a 16,17-double bond, are powerful hydroxylase/lyase inhibitors, useful for the above-stated purposes.

According to an important feature of the invention, there are provided compounds of the general formula



(1)

wherein X represents the residue of the A, B and C rings of a steroid, R represents a hydrogen atom or an alkyl group of 1 - 4 carbon atoms, R^{14} represents a hydrogen atom, a halogen atom or an alkyl group of 1 to 4 carbon atoms and each of the R^{15} substituents independently represents a hydrogen atom or an alkyl or alkoxy group of 1-4 carbon atoms, a hydroxy group or an alkylcarbonyloxy group of 2 to 5 carbon atoms or together represent an oxo or methylene group or R^{14} and one of the R^{15} groups together represent a double bond and the other R^{15} group represents a hydrogen atom or an alkyl group of 1 to 4 carbon atoms, and R^{16} represents a hydrogen atom, halogen atom, or an alkyl group of 1 to 4 carbon atoms, in the form of the free bases or pharmaceutically acceptable acid addition salts.

The term "steroid" herein includes any compound having the steroidal B and C rings, but in which all or part of the A ring is missing e.g. ring not closed (lacking the 2- or 3-position C-atom or both) or takes the form of a cyclopentane ring. It also includes azasteroids having a ring nitrogen atom in place of a ring carbon atom, especially in the A-ring such as in 4-azasteroids.

In general, the compounds of formula (1) are new and such compounds per se are included in the invention. However, certain of them have been disclosed as intermediates in the synthesis of certain steroids having a 3-pyridyl or 3-pyridonyl group in the 17β-position, see J. Wicha and M. Masnyk, Bulletin of the Polish Academy of Sciences: Chemistry 33 (1-2), 19-27 and 29-37 (1985). The first of these papers says that a 17β-side chain of the form -C=C-C=O or -C=C-C=N favours cardiotonic properties and describes the synthesis of 17β-(3-pyridyl)-14β-androst-4-ene-3β,14-diol, while the second uses this compound to prepare 17β-[3-pyrid-2(1H)onyl]-14β-androst-4-ene-3β,14-diol. Those final compounds differ from those of the present invention by having a saturated D-ring and the paper contains no test results. Insofar as certain compounds within formula (1) are known as intermediates in these syntheses, the invention extends to them only for use in therapy.

These are 17-(3-pyridyl)androsta-5,14,16-trien-3β-ol and 15α- and 15β-acetoxy-17-(3-pyridyl)androsta-5,16-dien-3β-ol and their 3-acetates. See also J Wicha. et. al., Heterocycles 20, 231-234 (1983) which is a preliminary communication of the first of the above two papers.

J. Wicha et. al., Bulletin of the Polish Academy of Sciences, Chemistry 22 (1-2), 75-83 (1984) have also described the preparation of 3β-methoxy-17β-(3-pyridyl)androstane and pyridone analogues thereof via the intermediate 3β-methoxy-17-(3-pyridyl) androst-16-ene. Accordingly, the invention extends to the latter compound only for use in therapy. A preliminary communication of this paper, by J. Wicha and M. Masynk, appeared in Heterocycles 16, 521-524 (1981).

The invention also includes pharmaceutical compositions comprising a compound of formula (1) in association with a pharmaceutically acceptable diluent or carrier.

Description of the preferred embodiments

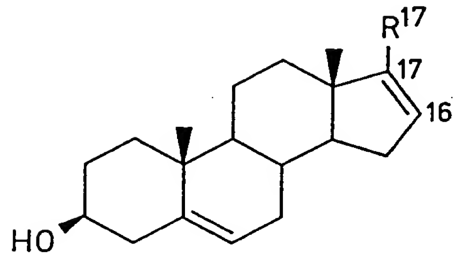
In the compounds of the invention the essential structural features comprise all of:

- a 3-pyridyl ring in the 17-position
- a ring double bond in the 16,17-position of the D-ring
- the 18-position methyl group

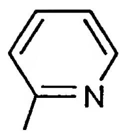
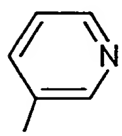
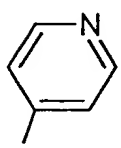
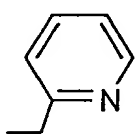
It is critical that the pyridine nitrogen atom be in the 3-position, not the 2- or 4-position. It is also critical that the pyridine ring be joined directly to the 17-carbon atom. This criticality is demonstrated by tests of inhibiting activity against hydroxylase and lyase (Table 1). The concentration of test compound required to achieve 50% inhibition of the enzyme is far greater for the 2-pyridyl, 4-pyridyl and 2-pyridylmethyl compounds tested than for the 3-pyridyl. The methods of determination were as described in the Examples hereinafter.

TABLE 1

Effect of variations in the 17-substitutent on inhibition of hydroxylase and lyase, demonstrating the criticality of the 17-substituent in this invention.



(2)

R^{17}	Type	IC_{50} (µM)	
		Lyase	Hydroylase
	2-Pyridyl (for comparison)	0.41	1.13
	3-pyridyl (present invention)	0.001	0.002
	4-pyridyl (for comparison)	2.0	6.0
	2-picolyl (for comparison)	>10	>10

Note: all the compounds of formula (2) tested were poor inhibitors of aromatase: IC_{50} >20 µM.

Elsewhere, the D-ring can have any other simple substituent. Certain simple substituents are defined in connection with the preferred general formula (1), but it will be appreciated that others could be substituted for those of formula (1). In the compounds of formula (1), R^{15} is preferably hydrogen or alkyl of 1 to 3 carbon atoms, R^{16} hydrogen, alkyl of 1 to 3 carbon atoms, fluorine, chlorine, bromine or iodine, and R hydrogen or methyl, especially in the 6-position of the pyridine ring.

The remainder of the molecule, designated "X" in formula (1), can be of any kind conventional in steroid chemistry or have any other feature known in steroids having anti-androgenic activity, for example the pyrazole or triazole ring, fused to the A ring at the 2- and 3- positions, disclosed in the above-cited Specification WO 92/00992, or oxazole ring fused in the same positions.

By way of example, X can represent the residue of
androstan-3α- or 3β-ol,
androst-5-en-3α- or 3β-ol,
androst-4-en-3-one,
androst-2-ene,
androst-4-ene,
androst-5-ene,
androsta-5,7-dien-3α or 3β-ol,
androsta-1,4-dien-3-one,
androsta-3,5-diene,
estra-1,3,5[10]-triene,
estra-1,3,5[10]-trien-3-ol,
5α-androstan-3-one,
androst-4-ene-3,11-dione,
6-fluoroandrost-4-ene-3-one or
androstan-4-ene-3,6-dione

each of which, where structurally permissible, can be further derivatised in one or more of the following ways:

- to form 3-esters, especially 3-alkanoates and -benzoates,
- to have one or more carbon to carbon ring double bonds in any of the 5,6-, 6,7- 7,8-, 9,11- and 11,12-positions
- as 3-oximes
- as 3-methylenes
- as 3-carboxylates
- as 3-nitriles
- as 3-nitros
- as 3-desoxy derivatives

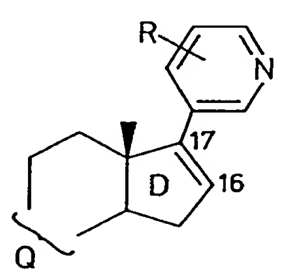
- to have one or more hydroxy, halo, C_{1-4}-alkyl, trifluoromethyl, C_{1-4}-alkoxy, C_{1-4}-alkanoyloxy, benzoyloxy, oxo, methylene or alkenyl substituents in the A, B or C-ring
- to be 19-nor.

Preferred C_{1-4}-alkyl and alkoxy groups are methyl and ethoxy.
Preferred C_{1-4}-alkanoyloxy groups are acetoxy and propanoyloxy.
Preferred halo groups are fluoro, bromo and chloro and the preferred substitution position is the 6-position.

The substituents include, for instance, 2-fluoro, 4-fluoro, 6-fluoro, 9-fluoro, 3-trifluoromtheyl, 6-methyl, 7-methyl, 6-oxo, 7-oxo, 11-oxo, 6-methylene, 11-methylene, 4-hydroxy, 7-hydroxy, 11-hydroxy or 12-hydroxy in any appropriate epimeric form and, subject to structural compatibility, in any combination of two or more such groups.

Compounds which are likely to be unstable are considered excluded from consideration. Such compounds will be evident to steroid chemists. Compounds having esoteric substituents likely to interfere with the stereochemical alignment of the steroid molecule with the enzymes to be inhibited, by virtue of steric or electronic distribution effects, are to be avoided. For example, a 2,3,5,6-tetrafluoro-4-trifluoromethylphenoxy substituent in the 3-position is not recommended. Androst-5-en-3β-ol having such an ether substituent in place of the 3β-hydroxy group has been shown to be a very poor inhibitor for lyase and hydroxylase.

The currently preferred compounds of formula (1) are those which are saturated and unsubstituted at the 11- and 12-positions and which therefore are of the general formula (3):



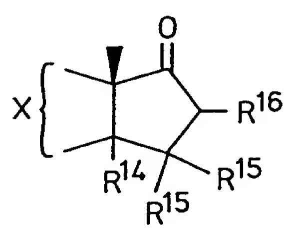
(3)

wherein Q represents the residue of A, B and C rings of a steroid, and R is a hydrogen atom or an alkyl group of 1-4 carbon atoms.

Specifically preferred compounds of the invention comprise
17-(3-pyridyl)androsta-5,16-dien-3β-ol,
17-(3-pyridyl)androsta-3,5,16-triene,
17-(3-pyridyl)androsta-4,16-dien-3-one,
17-(3-pyridyl)estra-1,3,5[10],16-tetraen-3-ol,
17-(3-pyridyl)-5α-androst-16-en-3α-ol
and their acid addition salts and 3-esters.

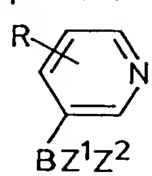
Other notable compounds of the ivention comprise
17-(3-pyridyl)-5α-androst-16-en-3-one,
17-(3-pyridyl)-androsta-4, 16-diene-3,11-dione,
17-(3-pyridyl)-androsta-3, 5, 16-trien-3-ol,
6α-and 6β-fluoro-17-(3-pyridyl)androsta-4, 16-dien-3-one
17-(3-pyridyl)androsta-4,16-dien-3, 6-dione,
17-[3-(6-methyl pyridyl)]androsta-5, 16 dien-3β-ol
3α-triflurομethyl-17-(3-pyridyl)androsta 16 en-3β-ol
and their acid addition salts and 3-esters.

The compounds of formula (1) can be prepared by a method which is in itself novel and inventive. Starting from a 17-oxo compound of general formula (4):



(4)

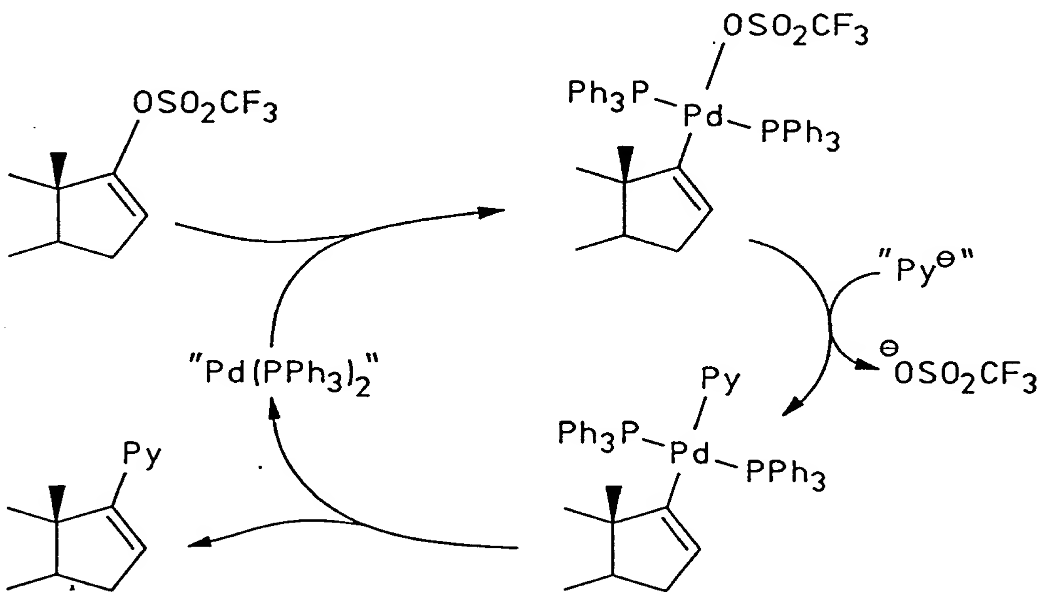
wherein X, R^{14}, R^{15} and R^{16} are as defined above and any other oxo groups and hydroxy groups in the molecule are first appropriately protected, the method comprises replacing the 17-hydroxy group of compound (4) in its enol form by a leaving group (L) which is capable of being replaced by a 3-pyridyl group in a palladium complex-catalysed cross-coupling reaction with a pyridyl-substituted boron compound of formula (5):



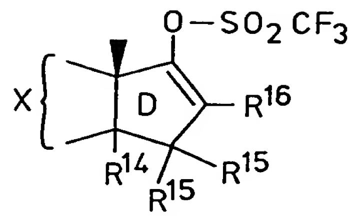
(5)

wherein Z^1 and Z^2 independently represent hydroxy or alkoxy or alkyl of 1-3 carbon atoms each, preferably ethyl or methoxy, and R is as defined above and carrying out said cross-coupling reaction.

The palladium complex-catalysed cross-coupling reaction of the 17-substituted steroid with the boron compound is believed to involve the steps indicated in the following illustrative reaction scheme (Py = 3-pyridyl). The pyridyl anionic species is provided by the boron compound.

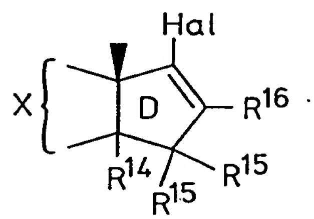


The replacement of the 17-enol group can be, for example, to form a 16,17-ene trifluoromethanesulphonate of formula (6):



(6)

or a 17-iodo or bromo-16,[17]-ene of formula (7):



(7)

(Hal = I or Br)

Compounds of formula (6) can be prepared by reacting the 17-oxo compound of formula (4) with an enol ester-forming trifluoromethanesulphonic acid derivative such as the anhydride, see S. Cacchi, E. Morera and G. Ortar, Tetrahedron Letters, 25, 4821 (1984). The 17-oxo compound can be considered notionally to exist in the enol form, the reaction being one of esterification of the enol.

Compounds of formula (7) can be prepared by first hydrazinating the 17-oxo compounds of formula (4) by a standard method to form the 17-hydrazone, which is then reacted with bromine or iodine in the presence of an amine or guanidine base, see D. Barton, G. Bashiardes and J. Fourrey, Tetrahedron Letters, 24, 1605 (1983).

For the preparation of the 17-position derivatives of formula (6) or (7) any necessary protection of other groups in the molecule is first carried out. For example hydroxyl groups are conveniently protected as their acetates, whilst the 3-oxo group of steroids can be selectively protected as their perfluorotolyl enol ethers, see M. Jarman and R. McCague, J.Chem.Soc. Perkin Trans. 1, 1129 (1987).

The 17-position derivative is then reacted with the boron compound of formula (5) using as catalyst a palladium(0) phosphine complex, for example tetrakis(triphenylphosphine)palladium(0), or a palladium (II) phosphine complex which is reducible _in situ_ to a palladium(0) phosphine species, especially bis(triphenylphosphine)palladium (II) chloride.

Further compounds of the invention can be prepared by standard steroid to steroid inter-conversion chemistry, so long as the D-ring chemical structure is not affected thereby. If the D-ring structure is likely to be affected, it would usually be necessary to prepare the desired compound _de novo_, i.e. by choosing the appropriate starting compound of formula (4), protected if necessary, and carrying out the reactions of 17-substitution of the enol and cross-coupling with the boron compound as described above.

By way of example, the 3-esters of a steroid 3-ol with an alkanoic acid of 1 to 6 carbon atoms, or a cycloalkanoic acid or aralkanoic acid such as phenylacetic or phenylpropionic acid, an aroic acid such as benzoic acid, or other simple organic acid such as methanesulphonic acid, can be converted into the 3-ol or the 3-ol to the 3-ester. Other examples of simple conversions which would not affect the D-ring structure are

i) Oppenauer oxidation using cyclohexanone and aluminium isopropoxide to convert 3-hydroxy to 3-oxo steroids and notably $\Delta^{5,6}$-3-hydroxy to $\Delta^{4,5}$-3-oxo steroids;

ii) Wittig olefination to convert oxo groups to methylene groups [D. D. Evans _et al_., J. Chem. Soc., 4312-4317, (1963)];

iii) Oxidation of Δ^5-3β-hydroxy to Δ^4-3,6-dione steroids using _N_-methylmorpholine _N_-oxide and tetra-_n_-propylammonium perruthenate catalyst [M. Moreno _et al_., Tetrahedron Letters, _32_, 3201-3204, (1991)];

iv) 6-Methylenation of Δ^4-3-oxo steroids using formaldehyde dimethylacetal [K. Annen _et al_., Synthesis, 34-40 (1982)];

v) Conversion of Δ^4-3-oxo to 4,4-dimethyl-Δ^5-3-oxo, $\Delta^{1,4}$-3-oxo, $\Delta^{1,4,6}$-3-oxo, 7α-methyl-Δ^4-3-oxo, $\Delta^{4,6}$-3-oxo, 6-chloro-Δ-^{4,6}-3-oxo, $\Delta^{2,4}$-2,3-isoxazole, 6α-methyl-Δ^4-3-oxo and Δ^4-3-desoxy; Δ^5-3β-ol to 5α-fluoro-6-oxo, 5α,6,6-trifluoro, 6,6-difluoro and 6α-fluoro-Δ^4-3-oxo; and 11-oxo to 11-hydroxy and $\Delta^{9,11}$ steroids [D. Lednicer and L. A. Mitscher, The Organic Chemistry of Drug Synthesis, 1s. 2 and 3, Wiley (1980 and 1984)] or

v) Electrophilic fluorination of steroids using N-fluoropyridinium reagents [T. Umenoto <u>et</u> <u>al</u>., Organic Synthesis <u>69</u>, 129 - 143 (1990)].

The compounds of formula (1) may be prepared as salts, e.g. the hydrochloride and converted to the free base form and thereafter to such other conventional pharmaceutically acceptable salts as acetates, citrates and lactates, as may seem appropriate.

The present invention also provides a pharmaceutical composition which comprises a therapeutically effective amount of a compound of the invention, in association with a therapeutically acceptable carrier or diluent. The composition of the invention can, for example, be in a form suitable for parenteral (e.g. intravenous, intramuscular or intracavital), oral, topical or rectal administration. Particular forms of the composition may be, for example, solutions, suspensions, emulsions, creams, tablets, capsules, lipsomes or micro-reservoirs, especially compositions in orally ingestible or sterile injectable form. The preferred form of composition contemplated is the dry solid form, which includes capsules, granules, tablets, pills, boluses and powders. The solid carrier may comprise one or more excipients, e.g. lactose, fillers, disintegrating agents, binders, e.g. cellulose, carboxymethylcellulose or starch or anti-stick agents, e.g. magnesium stearate, to prevent tablets from adhering to tabletting equipment. Tablets, pills and boluses may be formed so as to disintegrate rapidly or to provide slow release of the active ingredient.

Where national patent law permits, the present invention also includes a method of treating androgen- and oestrogen-dependent disorders, especially tumours, and most especially prostatic tumours, in the mammalian body, which comprises administering a compound of the invention to a mammalian patient in a therapeutically effective dose, e.g. in the range 0.001-0.04 mmole/kg body weight, preferably 0.001-0.01 mmole/kg, administered daily or twice daily during the course of treatment. This works out (for humans) at 20-800 mg/patient per day. Alternatively the invention includes the compounds of the invention for use in said treatment and their use in the manufacture of medicaments for that purpose. The preferred use is in treating prostatic cancer. Another use is in treating breast cancer.

The following Examples illustrate the invention.

Example 1.

(a) 3β-Acetoxyandrosta-5,16-dien-17-yl trifluoromethanesulphonate

To a stirred solution of dehydroepiandrosterone-3-acetate (24.8g, 75 mmol) in dry dichloromethane (500 ml) containing 2,6-di-*t*-butyl-4-methylpyridine (18.5g, 90 mmol) was added trifluoromethanesulphonic anhydride (12.6 ml, 75 mmol). After 12h the mixture was filtered and washed with water (50 ml), dried ($MgSO_4$), and the solvent evaporated. Chromatography, on elution with light petroleum-dichloromethane (6:1), gave firstly androsta-3,5,16-trien-17-yl trifluoromethanesulphonate (3.02g, 10%) as an oil. ^{1}H-NMR($CDCl_3$) *inter alia* δ 0.99 (3H,s,18-C$\underline{H}_3$), 1.02(3H,s,19-C$\underline{H}_3$), 5.39(1H,m,6-$\underline{H}$), 5.59(1H,m,16-$\underline{H}$), 5.62(1H,m,3-$\underline{H}$), 5.93(1H,dm,J 9.4Hz,4-$\underline{H}$); MS *m/z* 402(M^+). Further elution with light petroleum-dichloromethane (3:1) afforded the *title compound* (20.1g, 58%) which crystallised from hexane, m.p. 75-76°C. ^{1}H-NMR($CDCl_3$) *inter alia* δ 1.00(3H,s,18-C$\underline{H}_3$), 1.06(3H, s,19-C$\underline{H}_3$), 2.04(3H,s,C$\underline{H}_3CO_2$), 4.59(1H,m,3α-$\underline{H}$), 5.39(1H,dm,J 4.9 Hz,6-$\underline{H}$), 5.58(1H,m,16-$\underline{H}$). Anal. Calcd: C,57.13; H,6.32; s,6.93. Found: C,57.29; H,6.31; S,6.96%.

(b) 3β-Acetoxy-17-(3-pyridyl)androsta-5,16-diene

Diethyl(3-pyridyl)borane (3.38g, 23 mmol) was added to a stirred solution of 3β-acetoxyandrosta-5,16-dien-17-yl trifluoromethanesulphonate (6.94g, 15 mmol) in THF (75 ml) containing bis(triphenylphosphine)palladium(II) chloride (0.105g, 0.15 mmol). An aqueous solution of sodium carbonate (2M, 30 ml) was then added and the mixture heated, with stirring, by an oil bath at 80°C for 1h, and allowed to cool. The mixture was partitioned between diethyl ether and water, the ether phase was dried (Na_2CO_3), filtered through a short plug of silica, and concentrated. Chromatography, on elution with light petroleum-diethyl ether (2:1), afforded the title compound (4.95g, 84%) which crystallised from hexane, m.p. 144-145°C, ^{1}H-NMR($CDCl_3$) inter alia δ 1.05(3H,s,19-C$\underline{H}_3$), 1.08(3H,s,18-C$\underline{H}_3$), 2.04(3H,s,C$\underline{H}_3$$CO_2$), 4.60(1H,m,3α-$\underline{H}$), 5.42(1H,dm, J 4.7Hz,6-$\underline{H}$), 5.99(1H,m,16-$\underline{H}$), 7.23(1H,m,Py 5-$\underline{H}$) 7.65(1H,m,Py 4-$\underline{H}$), 8.46(1H,m,Py 6-$\underline{H}$), 8.62(1H,m,Py 2-$\underline{H}$). Anal. Calcd: C, 79.75; H, 8.50; N, 3.58. Found: C, 79.78; H, 8.52; N, 3.54%.

Example 2.

17-(3-Pyridyl)androsta-5,16-dien-3β-ol

To a solution of 3β-acetoxy-17-(3-pyridyl)androsta-5,16-diene (4.90g, 12.5 mmol) in methanol (50 ml) was added an aqueous solution of sodium hydroxide (10% w/v, 10 ml) and the mixture heated, with stirring, on an oil bath at 80°C for 5 min., then allowed to cool. The mixture was poured into water, neutralised with hydrochloric acid (1M), rebasified with saturated sodium bicarbonate solution, and extracted with hot toluene (3 x 100 ml). The toluene extracts were combined, dried (Na_2CO_3), and concentrated. Chromatography, on elution with toluene-diethyl ether (2:1) afforded the title compound (3.45g, 79%) which crystallised from toluene, mp 228-229°C; ^{1}H-NMR ($CDCl_3$ inter alia δ 1.05(3H,s,19-C$\underline{H}_3$), 1.07(3H,s,18-C$\underline{H}_3$), 3.54(1H,m,3α-$\underline{H}$), 5.40(1H,dm, J 5.0 Hz, 6-$\underline{H}$), 5.99(1H,m,16-$\underline{H}$), 7.22(1H,m,Py5-$\underline{H}$), 7.65(1H,m,Py 4-$\underline{H}$), 8.46(1H,m,Py 6-$\underline{H}$), 8.62(1H,m,Py 2-$\underline{H}$). Anal. Calcd: C, 82.47; H, 8.94; N, 4.01. Found: C, 82.40; H, 8.91; N, 3.97%.

Example 3.

17-(3-Pyridyl)androsta-3,5,16-triene

The method followed that described in Example 1, using in step (b) diethyl(3-pyridyl)borane (0.88g, 6.0 mmol), androsta-3,5,16-trien-17-yl trifluoromethanesulphonate (2.01g, 5.0 mmol), prepared in step (a), THF (25 ml), bis(triphenylphosphine)-palladium(II) chloride (35 mg, 0.05 mmol), and aqueous sodium carbonate (2M, 10 ml). Chromatography, on elution with dichloromethane, afforded the title compound (1.39g, 84%) which crystallised from hexane, m.p. 110-112°C. ^{1}H-NMR ($CDCl_3$) *inter alia* δ 1.02(3H,s,19-CH_3), 1.07(3H,s,18-CH_3), 5.44(1H,m,6-*H*), 5.61(1H,m,3-*H*), 5.95(1H,dm, J 9.8Hz, 4-*H*), 6.01(1H,m,16-*H*), 7.23(1H,m,Py 5-*H*), 7.66(1H,m,Py 4-*H*), 8.46(1H,m,Py 6-*H*), 8.63(1H,m,Py 2-*H*); MS *m/z* 331 (M^+).

Example 4

(a) 3-[2,3,5,6-tetrafluoro-4-(trifluoromethyl)phenoxy]androsta-3,5,16-trien-17-yl trifluoromethanesulphonate

The method followed that described in Example 1(a) but using 3-[2,3,5,6-tetrafluoro-4-(trifluoromethyl)phenoxy]androsta-3,5-dien-17-one (5.03g, 10 mmol), prepared as described in M. Jarman and R. McCague, J. Chem. Soc., Perkin Trans. 1, 1129 (1987), dichloromethane (80 ml), 2,6-di-*t*-butyl-4-methylpyridine (2.87g, 14 mmol), and trifluoromethanesulphonic anhydride (1.85 ml, 11 mmol). Chromatography, on elution with light petroleum-dichloromethane (10:1), afforded the title compound (1.93g, 30%) which crystallised from ethanol, m.p. 106-107°C. ^{1}H-NMR ($CDCl_3$) *inter alia* δ 1.02(6H,s,18 and 19-CH_3), 5.16(1H,s,4-*H*), 5.28(1H,m,6-*H*), 5.59(1H,m,16-*H*); MS *m/z* 634 (M^+).

(b) 3-[2,3,5,6-tetrafluoro-4-(trifluoromethyl)phenoxy]-17-(3-pyridyl)androsta-3,5,16-triene

The method essentially followed that of Example 1(b) but using diethyl(3-pyridyl)borane (0.44g, 3.0 mmol), 3-[2,3,5,6-tetrafluoro-4-(trifluoromethyl)phenoxy]androsta-3,5,16-trien-17-yl trifluoromethanesulphonate (1.27g, 2.0 mmol), THF (10 ml),

bis(triphenylphosphine)palladium(II) chloride (70mg, 0.1 mmol), and aqueous sodium carbonate (2M, 5 ml). Chromatography, on elution with light petroleum-diethyl ether (3:1), afforded the title compound (0.82g, 73%) which crystallised from hexane, m.p. 166.0-166.5°C. ^{1}H-NMR ($CDCl_3$) *inter alia* δ 1.05(3H,s,19-CH_3), 1.07(3H,s,18-CH_3), 5.18(1H,s,4-H), 5.32(1H,m,6-H), 6.01(1H,m,16-H), 7.23(1H,m,Py 5-H), 7.66(1H,m,Py 4-H), 8.47(1H,m,Py 6-H), 8.63(1H,m,Py 2-H). Anal. Calcd: C, 66.07; H, 5.01; N, 2.49; F, 23.60. Found: C, 65.97; H, 5.02; N, 2.47; F, 23.41%.

(c) 17-(3-Pyridyl)androsta-4,16-dien-3-one

To a solution of 3-[2,3,5,6-tetrafluoro-4-(trifluoromethyl)-phenoxy]-17-(3-pyridyl)androsta-3,5,16-triene (0.423g, 0.75 mmol) in THF (5 ml) was added ethanol (5 ml) followed by aqueous hydrochloric acid (1M, 5 ml) and the mixture heated, with stirring, by an oil bath at 65°C for 48h and allowed to cool. The mixture was poured into water (20 ml), neutralised with aqueous sodium hydroxide (1M), and extracted with diethyl ether (3 x 30 ml). The ether extracts were combined, dried (Na_2CO_3), and concentrated. Chromatography, on elution with diethyl ether, afforded the title compound (185mg, 71%) which crystallised from diethyl ether, m.p. 148-150°C. IR νmax 1674 cm^{-1}; ^{1}H-NMR($CDCl_3$) *inter alia* δ 1.07(3H,s,18-CH_3), 1.24(3H,s,19-CH_3), 5.76(1H,s,4-H), 5.99(1H,m,16-H), 7.23(1H,m,Py 5-H), 7.64(1H,m,Py 4-H), 8.47(1H,m,Py 6-H), 8.62(1H,m,Py 2-H); MS *m/z* 347 (M^+).

Example 5

(a) 3-Acetoxyestra-1,3,5[10],16-tetraen-17-yl trifluoromethanesulphonate

The method followed that described in Example 1(a), but using oestrone-3-acetate (4.69g, 15 mmol), dichloromethane (120 ml), 2,6-di-*t*-butyl-4-methylpyridine (4.00g, 19.5 mmol), and trifluoromethanesulphonic anhydride (2.8 ml, 16.5 mmol). Chromatography, on elution with light petroleum-dichloromethane (3:1), afforded the title compound (5.21g, 78%). ^{1}H-NMR($CDCl_3$)

inter alia δ 1.00(3H,s,18-C$\underline{H}_3$), 2.29(3H,s,C$\underline{H}_3$$CO_2$), 5.62(1H,m,16-$\underline{H}$), 6.81(1H,m,Ar$\underline{H}$), 6.85(1H,m,Ar$\underline{H}$), 7.26(1H,m,Ar$\underline{H}$). Anal. Calcd. for $C_{21}H_{23}O_5F_3S_1.½H_2O$: C, 55.62; H, 5.34. Found: C, 55.58: H, 5.14%.

(b) 3-Acetoxy-17-(3-pyridyl)estra-1,3,5[10],16-tetraene

The method followed that described in Example 1(b), but using diethyl(3-pyridyl)borane (1.65g, 11.2 mmol), 3-acetoxyestra-1,3,-5[10],16-tetraen-17-yl trifluoromethanesulphonate (3.56g, 8.0 mmol), THF (40 ml), bis(triphenylphosphine)palladium(II) chloride (56mg, 0.08 mmol), and aqueous sodium carbonate (2M, 15 ml).

Chromatography, on elution with light petroleum-diethyl-ether (2:1) afforded the *title compound* (2.40g, 80%). ^{1}H-NMR($CDCl_3$) *inter alia* δ 1.04(3H, s,18-C$\underline{H}$), 2.29(3H, s, C$\underline{H}_3$$CO_2$), 6.03(1H,m,16-$\underline{H}$), 6.82(1H,m,Ar$\underline{H}$), 6.85(1H,m,Ar$\underline{H}$), 7.24(1H,m,Py 5-$\underline{H}$), 7.29(1H,m,Ar$\underline{H}$), 7.69(1H,m,Py 4-$\underline{H}$), 8.48(1H,m,Py 6-$\underline{H}$), 8.65(1H,m,Py 2-$\underline{H}$); MS *m/z* 373. ($M^+$).

Example 6

17-(3-Pyridyl)estra-1,3,5[10],16-tetraen-3-ol

The method followed that described in Example 2, but using 3-acetoxy-17-(3-pyridyl)estra-1,3,5[10],16-tetraene (2.36g, 6.3 mmol), methanol (40 ml), aqueous sodium hydroxide (10% w/v, 5 ml), and the mixture was heated at 80°C for 10 min. Chromatography, on elution with toluene-methanol (8:1), afforded the *title compound* (1.40g, 67%) which crystallised from toluene, m.p. 256-258°C: ^{1}H-NMR(DMSO) *inter alia* δ 1.01(3H,s,18-C$\underline{H}_3$), 6.15(1H,m,16-$\underline{H}$), 6.47(1H,m,Ar$\underline{H}$), 6.52(1H,m,Ar$\underline{H}$), 7.04(1H,m,Ar$\underline{H}$), 7.35(1H,m,Py 5-$\underline{H}$), 7.79(1H,m,Py 4-$\underline{H}$), 8.45(1H,m,Py 6-$\underline{H}$), 8.62(1H;m,Py 2-$\underline{H}$). Anal. Calcd: C, 83.34; H, 7.60; N, 4.23. Found: C, 83.39; H, 7.78; N, 4.06%.

Example 7

3α-Acetoxy-17-(3-pyridyl)-5α-androst-16-ene

The method followed that described in Example 1, using in

step (b) diethyl(3-pyridyl)borane (1.41g, 9.6 mmol), 3α-acetoxy-5α-androst-16-en-17-yl trifluoromethanesulphonate (3.44g, 7.4 mmol), prepared from the 3α-acetoxy-5α-androstan-17-one by the method of step (a), THF (40 ml), bis(triphenylphosphine)-palladium(II) chloride (52 mg, 0.07 mmol), and aqueous sodium carbonate (2M, 15 mmol). Chromatography, on elution with light petroleum-diethyl ether (2:1), afforded the title compound (2.39g, 82%), ^{1}H-NMR ($CDCl_3$) *inter alia* δ 0.85(3H,s,19-CH_3), 1.01(3H,s,18-CH_3), 2.06(3H,s,CH_3CO_2), 5.02(1H,m,3β-H), 6.00(1H,m,16-H), 7.24(1H,m,Py 5-H), 7.68(1H,m,Py 4-H), 8.47(1H,m,Py 6-H), 8.63(1H,m,Py 2-H); MS *m/z* 393 (M^+).

Example 8

17-(3-Pyridyl)-5α-androst-16-en-3α-ol

The method followed that described in Example 2, but using 3α-acetoxy-17-(3-pyridyl)-5α-androst-16-ene (2.33g, 5.9 mmol), methanol (40 ml), aqueous sodium hydroxide (10% w/v, 8 ml), and the mixture was heated at 80°C for 20 min. Chromatography, on elution with toluene-methanol (40:1), afforded the title compound (1.62g, 78%) which crystallised from toluene, m.p. 198-199°C; ^{1}H-NMR($CDCl_3$) *inter alia*. δ 0.84(3H,s,19-CH_3), 1.00(3H,s,18-CH_3), 4.06(1H,m,3β-H), 5.97(1H,m,16-H), 7.21(1H,m,Py 5-H), 7.64(1H,m,Py 4-H), 8.45(1H,m,Py 6-H), 8.61(1H,m,Py 2-H). Anal. Calcd: C, 82.00; H,9.46; N,3.99. Found: C,81.78; H,9.47; N.3.96%.

Example 9

17-(3-Pyridyl)-5α-androst-16-en-3-one

From a solution of 17-(3-Pyridyl)-5α-androst-16-en-3α-ol (1.05g, 3.0 mmol) in dry toluene (60ml) and cyclohexanone (10ml) was distilled off part of the solvent (20ml) to eliminate moisture. After allowing to cool to 90°C, aluminium isopropoxide (1.02g, 5.0 mmol) was added and the mixture heated under reflux for 90 min. then allowed to cool. The mixture was diluted with diethyl ether (250 ml), washed with aqueous trisodium citrate (15% w/v; 2 x 30ml), dried (Na_2CO_3), and concentrated. Chromatography, on

elution with toluene-methanol (40:1), afforded the title compound (0.90g, 86%) which crystallised from toluene, m.p. 190-192°C. IR νmax 1713 cm^{-1}; ^{1}H-NMR ($CDCl_3$) _inter alia_ δ 1.04 (3H,s,19-$\underline{CH_3}$), 1.07 (3H,s,18-$\underline{CH_3}$), 5.98 (1H,M,16-_H_), 7.22 (1H,m,Py 5-_H_), 7.64 (1H,m,Py 4-_H_), 8.46 (1H,m,Py 6-_H_), 8.61 (1H,m,Py 2-_H_); MS _m/z_ 349 (M+). Anal. Calcd: C,82.47; H,8.94; N,4.01. Found: C,82.00; H,8.94; N,3.84%

Example 10

a) 3-(tert-Butyldimethylsiloxy)androsta-3,5-diene-11,17-dione

To a solution of adrenosterone (6.0g, 20 mmol) in dry dichloromethane (120ml) was added triethylamine (8.4ml, 60 mmol) followed by _tert_-butyldimethylsilyl trifluoromethanesulfonate (5.0ml, 22 mmol) and the mixture stirred at room temperature for 3h. The dichloromethane was evaporated and the residue redissolved in diethyl ether (100ml), then allowed to stand for 30 min, after which time an oil separated. The ether phase was decanted off the oil and the solvent evaporated to give the _title compound_ which was used directly in step (b). IR νmax 1705, 1747 cm^{-1}; ^{1}H-NMR($CDCl_3$) _inter alia_ δ0.12 (6H,s,$\underline{Me_2}$Si), 0.85 (3H,s,18-$\underline{CH_3}$), 0.92 (9H,s,$^{t}\underline{Bu}$Si) 1.17(3H,s,19-$\underline{CH_3}$), 4.73 (1H,dm, J 6.9Hz, 6-_H_), 5.36 (1H,m,4-_H_).

b) 13-(tert-Butyldimethylsiloxy)-11-oxo-androsta-3,5,16-trien-17-yl trifluoromethanesulfonate

To a solution of the product from step (a) in dry THF (100ml), cooled to -78°C, was added a freshly prepared solution of lithium diisopropylamide [prepared by adding _n_-butyllithium (1.6M; 13.8ml, 22 mmol) in hexane to a solution of diisopropylamine (3.1ml, 22 mmol) in dry THF (25ml) at -18°C] and the resultant yellow solution stirred at -78°C for 30 min. A solution of _N_-phenyltrifluoromethanesulfonimide (7.15g, 20 mmol) in dry THF (20ml) was then added and after an additional 1h. at -78°C was allowed to reach ambient temperature. The reaction mixture was poured into water (200 ml) and extracted with diethyl ether (2 x 200ml), the combined ether extracts were washed with water

(20ml), dried Na_2CO_3), and concentrated to give the title compound which was used directly in step (c). IR νmax 1710 cm^{-1}, ^1H-NMR ($CDCl_3$) inter alia δ0.13 (6H,S,Me_2Si), 0.92 (9H,s,^{t}Bu Si), 1.35 (6H,2s,18-CH_3 and 19-CH_3), 4.75 (1H,m,6-H) 5.38 (1H,s,4-H), 5.68 (1H,m,16-H).

c) 3-(tert-Butyldimethylsiloxy)-17-(3-pyridyl)androsta-3,5,16-trien-11-one

The method essentially followed that described in Example 1 (b), but using the 13-(tert-butyldimethylsiloxy)-11-oxo-androsta-3,5,16-trien-17-yl trifluoromethanesulfonate from step (b), diethyl (3-pyridyl)borane (3.53g, 24 mmol), THF (100ml), bis(triphenylphosphine)palladium (II) chloride (280mg, 0.4 mmol), and aqueous sodium carbonate (2M;50ml). Following work-up as described in Example 1 (b) the title compound was obtained, which was used directly in step (d). IR νmax 1705 cm^{-1}, ^1H-NMR ($CDCl_3$) inter alia δ0.13 (3H,s,Me_2Si), 0.93 (9H,s,^{t}BuSi), 0.99 (3H,s,18-CH_3), 1.18 (3H,s,19-CH_3), 4.75 (1H,m,6-H) 5.37 (1H,m,4-H), 6.09 (1H,m,16-H), 7.26 (1H,m,Py 5-H), 7.62 (1H,m,Py 4-H), 8.50 (1H,m,Py 6-H), 8.60 (1H,m,Py 2-H). MS m/z 475 (M+).

d) 17-(3-Pyridyl)androsta-4,16-diene-3,11-dione

To a solution of the product from step (c) in wet THF (60ml) was added a solution of tetrabutylammonium fluoride (1.0M; 10ml, 10 mmol) in THF, and the mixture stirred at room temperature for 12 h. The mixture was partitioned between diethyl ether and water basified with saturated aqueous sodium bicarbonate, the ether phase isolated, dried (Na_2CO_3), and concentrated. Chromatography, on elution with diethyl ether, afforded the title compound (4.30g, 60% overall yield from adrenosterone) which crystallised from diethyl ether, m.p. 181-183°C.

IR νmax 1669, 1703 cm^{-1}' ^1H-NMR($CDCl_3$) inter alia δ1.02 (3H,s, 18-CH_3), 1.45 (3H,s,19-CH_3), 5.76 (1H,(1H,s,Py 4-H), 6.08

(1H,m,16-H) 7.24 (1H,m,Py 5-H), 7.59 (1H,m,Py 4-H),8.50 (1H,m,Py 6-H), 8.59 (1H,m,Py 2-H). MS m/z 361 (M+). Anal Calcd: C, 79.74; H,7.53: N,3.88. Found: C,79.58; H,7.57; N,3.89%.

Example 11

3-Acetoxy-17-(3-pyridyl)androsta-3,5,16-triene

17-(3-pyridyl)androsta-4,16-dien-3-one (174 mg, 0.50 mmol) was dissolved in isopropenyl acetate (2 ml). p-Toluenesulfonic acid (130 mg, 0.68 mmol) was then added and the mixture heated at 80°C for 12h. After allowing to cool the mixture was poured into diethyl ether, washed with saturated aqueous sodium bicarbonate, dried (Na_2CO_3) and concentrated. Chromatography on elution with light petroleum - diethyl ether (1:1), afforded the title compound (86 mg, 44%), IR νmax 1755 cm^{-1}, ^{1}H-NMR ($CDCl_3$) inter alia δ1.05 (6H,s,18-CH_3 and 19-CH_3), 2.15 (3H,s,COCH_3) 5.44 (1H,m,6-H), 5.72(1H,m,4-H), 6.00 (1H,m,16-H), 7.25 (1H,m,Py 5-H), 7.66 (1H,m,Py 4-H), 8.47 (1H,M,Py 6-H), 8.63 (1H,m,Py 2-H). MS m/z 389 (M+).

Example 12

6β-Fluoro-17-(3-pyridyl)androsta-4,16-dien-3-one

and

Example 13

6α-Fluoro-17-(3-pyridyl)androsta-4,16-dien-3-one

To a solution of 3-acetoxy-17-(3-pyridyl)androsta-3,5,16-triene (80mg, 0.21 mmol) in dry dichloromethane (2ml) was added N-fluoropyridinium trifluoromethanesulfonate (180mg, 0.73 mmol) and the mixture heated under reflux for 12h. The mixture was diluted with diethyl ether (30ml), washed with dilute aqueous sodium hydroxide (0.5M; 2 x 5ml), dried Na_2CO_3), and concentrated. ^{1}H and ^{19}F-NMR at this stage showed the 6-fluorinated products were formed as a 3:2 mixture of the β and α-epimers. Chromatography, on elution with light petroleum-diethyl ether (1:2), gave firstly:- i) the title 6β-epimer (13mg), 17%) as white crystals,

m.p. 167-169°C. IR νmax 1684 cm^{-1}; ^{1}H-NMR ($CDCl_3$) <u>inter alia</u> δ1.11 (3H,s,18-<u>CH_3</u>), 1.37 (3H,s,19-<u>CH_3</u>), 5.06 (1H,dd, J_{H-H} 2.4 Hz, J_{H-F} 49Hz, 6α-<u>H</u>), 5.92 (1H,m,4-<u>H</u>), 6.01 (1H,m,16-<u>H</u>), 7.24 (1H,m,Py 5-<u>H</u>), 7.65 (1H,m,Py 4-<u>H</u>), 8.48 (1H,m,Py 6-<u>H</u>), 8.63 (1H,m,Py 2-<u>H</u>). ^{19}F-NMR δ -165.9 (dt, J_{H-F} 49 Hz, 6β-<u>F</u>). MS <u>m</u>/<u>z</u> 365 (M+).

Further elution afforded:-

ii) The title 6α-epimer (8mg, 11%) as white crystals, m.p. 167-169°C, IR νmax 1681 cm^{-1}; ^{1}H-NMR ($CDCl_3$) <u>inter alia</u> δ1.07 (3H,s,18-<u>CH_3</u>), 1.24 (3H,s,19-<u>CH_3</u>), 5.18 (1H,dm, J_{H-F} 48Hz, 6β-<u>H</u>), 5.98 (ZH,m,4-<u>H</u> and 16-<u>H</u>), 7.26 (1H,m,Py 5-<u>H</u>), 7.64 (1H,m,Py 4-<u>H</u>), 8.40 (1H,m,Py6-<u>H</u>), 8.63 (1H,m,Py 2-<u>H</u>). ^{19}F-NMR ($CDCl_3$) δ -183.9 (d, J_{H-F} 48 Hz, 6α-<u>F</u>). MS <u>m</u>/<u>z</u> 365 (M+).

<u>Example 14</u>

<u>17-(3-pyridyl)androsta-4,16-dien-3-one (via Oppenauer Oxidation)</u>

This Example illustrates a better method of preparing the compound already prepared in Example 4. The method followed that described in Example 9, but using 17-(3-pyridyl)androsta-5, 16-dien-3β-ol (1.05g, 3.0 mmol). Chromatography, on elution with toluene-methanol (20:1), afforded the <u>title compound</u> (0.85g, 82%), which crystallised from diethyl ether, m.p. 148-150°C. Spectroscopic data was identical to that given in Example 4(c).

Anal. Calcd: C,82.95; H,8.41; N,4.03

Found: C,83.00; H,8.50; N,3.99%

<u>Example 15</u>

<u>17-(3-pyridyl)androsta-4,16-dien-3-one oxime</u>

To a suspension of 17-(3-pyridyl)androsta-4,16-dien-3-one (125 mg, 0.36 mmol) in ethanol (2 ml) was added hydroxylamine hydrochloride (50mg, 0.72 mmol), followed by pyridine (0.2ml), and the mixture heated under reflux for 1h. then allowed to cool.

The solvent was evaporated and the crystalline product triturated under water, collected on a sinter, washed with cold water, and dried _in vacuo_ to give the _title oxime_ as a 1:1 mixture of _syn_ and _anti_ geometric isomers. ^{1}H-NMR ($CDCl_3$) _inter alia_ δ1.06 (3H,s,18-CH_3), 1.13 (3H,s,19-CH_3), 5.75 and 5.80 (1H,2m, isomeric 4-_H_), 6.01 (1H,m,16-_H_), 7.26 (1H,m,Py 5_H_), 7.68 and 7.88 (1H, 2m, isomeric Py 4-_H_), 8.48 and 8.53 (1H, 2m, isomeric Py 6-_H_), 8.63 (1H,m,Py 2-_H_). MS _m/z_ 362 (M+).

Example 16

17-(3-pyridyl)androsta-4,16-diene-3,6-dione

To a solution of 17-(3-pyridyl)androsta-5,16-dien-3β-ol (350mg, 1.0 mmol) in dry dichloromethane (10 ml) was added _N_-methylmorphine _N_-oxide (351mg, 3.0 mmol) followed by 400mg of freshly dried and powdered 4Å molecular sieves and the mixture stirred for 10 min. Tetrapropylammonium perruthenate catalyst (35mg), 0.1 mmol) was then added, the reaction flask placed in an ultrasonic bath, and the mixture irradiated whilst maintaining the temperature between 20-30°C for 2 h. The mixture was then filtered, diluted with diethyl ether, washed with water, dried (Na_2CO_3), and concentrated. Chromatography, on elution with diethyl ether - ethyl acetate (5:1), afforded the _title compound_ (26 mg, 7%) as white crystals m.p. 210-212°C. IR νmax 1680 cm^{-1}; ^{1}H-NMR ($CDCl_3$) _inter alia_ δ1.10 (3H,s,18-CH_3), 1.44 (3H,s,19-CH_3), 4.42 (1H,m,enolic 2-_H_), 5.84 (1H,s,4-_H_), 6.01 (1H,m,16-_H_), 7.24 (1H,m,Py 5-_H_), 7.65 (1H,m,Py 4-_H_), 8.45 (1H,m,Py 4-_H_), 8.45 (1H,m,Py 6-_H_), 8.60 (1H,m,Py 2-_H_). FAB-MS MS _m/z_ 362 (M+1).

Example 17

17-[3-(6-Methylpyridyl)]androsta-5,16-dien-3β-ol

To a suspension of 17-(3-pyridyl)androsta-5,16-dien-3β-ol (175mg, 0.5 mmol) in dry THF (3ml) was added dropwise a solution of methyllithium (1.4M; 0.9ml, 1.25 mmol) in diethyl ether to give a

yellow/green coloured solution. The mixture was stirred for 2h. at room temperature then poured into water, extracted with toluene, the organic phase dried (Na_2CO_3), and concentrated. Chromatography, on elution with light petroleum - diethyl ether (1:1), afforded the title compound (45mg, 25%) ^{1}H-NMR ($CDCl_3$) *inter alia* δ1.03 (3H,s,19-CH_3), 1.07 (3H,s,18-CH_3), 2.54 (3H,s,18-CH_3), 2.54 (3H,s,Py 6-CH_3), 3.48 (1H,m,3α-H), 5.38 (1H,m,6-H), 5.94 (1H,m,16-H), 7.08 (1H,d, J 8.0Hz, Py 4-H), 7.55 (1H,dd, J 2.2, 8.0Hz, Py 4-H), 8.50 (1H,d, J 2.0Hz,Py 2-H). MS *m/z* 363 (M+).

Example 18

3α-(Trifluoromethyl)-17-(3-pyridyl)androst-16-en-3β-ol

To a solution of 17-(3-pyridyl)androst-16-en-3-one (100 mg, 0.29 mmol) in THF (2ml) cooled to 0°C was added trifluoromethyltrimethylsilane (200µl, 1.3mmol) followed by tetrabutylammonium fluoride trihydrate (10 mg, 0.03 mmol). After 30 min., dilute aqueous hydrochloric acid (1M; 1ml.) was added and the mixture stirred at room temperature for 12h. The mixture was then basified with saturated aqueous sodium bicarbonate and extracted with diethyl ether. The three extracts were combined, dried (Na_2CO_3), and concentrated. Chromatography, on elution with light petroleum - diethyl ether (1:1), afforded the title compound (87mg, 73%) which crystallised from toluene, m.p. 192-193°C ^{1}H-NMR ($CDCl_3$) *inter alia* δ0.92 (3H,s,19-CH_3), 1.01 (3H,s,18-CH_3), 5.98 (1H,m,16-H), 7.22 (1H,m,Py 5-H), 7.64 (1H,m,Py 4-H),8.45 (1H,m,Py 6-H), 8.60 (1H,m,Py 2-H); ^{19}F-NMR ($CDCl_3$) δ -79.1 (s,3α-CF_3). MS *m/z* 419 (M+).

Anal. Calcd: C,71.57; H,7.69; N,3.34; F,13.59

Found: C,71.67; H,7.71; N,3.25; F,13.30%

Test results

(a) Preparation of testicular material

Human testes were obtained from previously untreated patients undergoing orchidectomy for prostatic cancer. The testes were decapsulated and stored in liquid nitrogen until use. A microsomal preparation was prepared essentially as described by S. E. Barrie et al., J. Steroid Biochem. 6, 1191-5, (1989). The material was then thawed, finely chopped, and homogenised in 0.25M sucrose (5ml/g wet weight) using a Potter homogeniser. The homogenate was centrifuged at 12000g for 30 min, and then the microsomes were pelleted by spinning the supernatant at 100,000g for 1hr. The pellet was washed by being resuspended in 0.25M sucrose and repelleted. The microsomal pellet was then resuspended in 50mM sodium phosphate pH 7.4/glycerol (3/1 v/v) and stored in aliquots in liquid nitrogen.

(b) Determination of 17α-hydroxylase

The basic assay mixture was EDTA (0.2mM), dithiothreitol (DTT; 1mM), NADPH (0.25mM), glucose 6-phosphate dehydrogenase (G6PDH; 6.25 μg/ml), $MgCl_2$ (1mM), glucose 6-phosphate (G6P; 10mM) and the substrate, ^{3}H-progesterone (3μM) in sodium phosphate (50mm), pH 7.4. The compounds under test were dissolved in 50% DMSO and the final concentrations of ethanol and DMSO were 1% each. The assay reaction was carried out for 1 hour and was terminated by the addition of 2 vols. of methanol-acetonitrile (2:1) containing approx. 100μM unlabelled progesterone, 17α-hydroxyprogesterone, androstenedione, testosterone, and 16α-hydroxyprogesterone. The last-mentioned steroid was added as it appeared that the human enzyme was capable of 16α-hydroxylation as well as 17α-hydroxylation.

The separation of the steroids by HPLC was by the method of S. E. Barrie et al., supra, except that the radioactivity in the peaks of interest has been monitored on-line by mixing the HPLC effluent 1:1 with Ecoscint A (National Diagnostics) scintillation

fluid, containing 25% acetonitrile, and passing the mixture through a Berthold LB506C radiochemical monitor. The hydroxylase activity was measured as the production of 17α-hydroxyprogesterone, androstenedione and testosterone.

(c) Determination of C_{17}-C_{20} lyase

The mixture was the same as described above for the 17α-hydroxylase except that the substrate was ^{3}H-17α-hydroxyprogesterone. The reaction was carried out for 1hr. and was stopped by the addition of 2 vols. of methanol/acetonitrile (2/1 containing approx. 100μM 17α-hydroxyprogesterone, androstenedione and testosterone.

The HPLC separation used for the lyase involved a mini-re-column "Uptight Guard Column" packed with PELL-ODS (pellicular octadecyl silica) and a 10cm. main column "Apex C18" column packed with 5μ APEX-CAT silica.

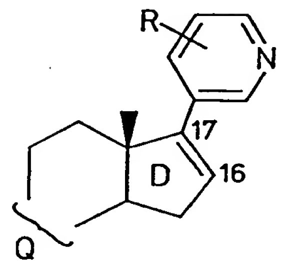
The eluant was 38:12:50 methanol:acetonitrile:water flowing at 1ml/min. The effluent was mixed 1:1 with Ecoscint A containing 5% methanol and 5% acetonitrile and the radioactivity was measured directly by a Berthold LB506C radiochemical detector. The lyase activity was measured as the production of androstenedione and testosterone.

(d) Calculation of IC_{50}.

The enzyme activity was measured in the presence of at least 4 concentrations of each compound, and the data were fitted by linear regression to the Dixon equation (M. Dixon, E.C. Webb, Enzymes, 2nd ed., Academic Press, New York, 1964). The IC_{50} was calculated from the slope. Results are shown in Table 2 below. These data can be used only in a comparative manner since the concentrations of enzyme and substrate used affect IC_{50} values.

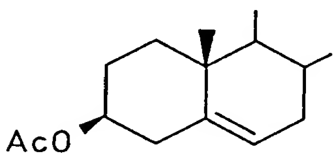
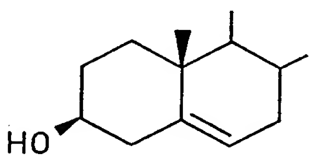
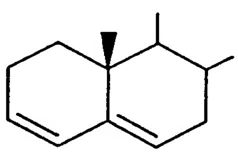
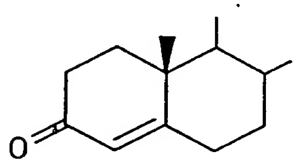
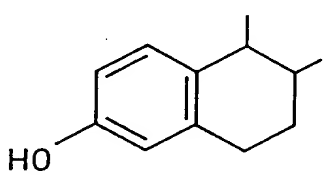
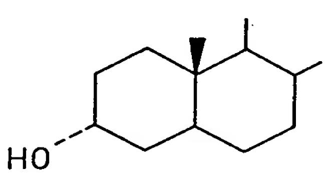
TABLE 2

Confirmation that variations in the A and B rings of compounds of the invention have little effect on inhibition of hydroxylase and lyase.



(3)

Compounds tested are of formula (3) wherein R = H:

Q		IC$_{50}$ (μM) Lyase	Hydroxylase
	(Ex. 1)	0.006	0.009
	(Ex. 2)	0.001	0.002
	(Ex. 3)	0.003	0.005
	(Ex. 4)	0.002	0.001
	(Ex. 6)	0.002	0.002
	(Ex. 8)	0.002	0.003

The comparative IC_{50} figures for Ketoconazole are 0.024 against lyase and 0.056 against hydroxylase.

Assay of aromatase activity

Aromatase activity was determined by the method of A. B. Foster et al., J. Med. Chem. 26, 50-54 (1983), using human placental microsomes. For the microsomes used, the Michaelis constant K_m for [1ß - ^{3}H] androstenedione was 0.039μM.

The compounds having a pregnenolone-like skeleton in the A and B rings, i.e. 3ß-acetoxy-17-(3-pyridyl)androsta-5,16-diene and its 3-alcohol of Examples 1 and 2, had IC_{50} > 20 μM. The compound having a progesterone-like skeleton in the A and B rings, i.e. 17-(3-pyridyl)-androsta-4,16-dien-3-one of Example 4 exhibited also aromatase inhibitory activity with IC_{50} = 1μM.

In vivo organ weight and endocrine test in mice

Male HWT mice, 12 weeks old, were treated daily for 2 weeks, with 5 animals per treatment group. The test compounds were the compound of Examples 1 and 4 (as representative of compounds of the invention having the pregenolone-like and progesterone-like skeletons respectively). Ketoconazole was also tested at three different doses. The test compounds were made up in 5% benzyl alcohol, 95% safflower oil, and were given i.p.. In addition to an untreated control group of animals, there was also a solvent control group which received the same volume of liquid as the test group (5ml/kg) but no test compound. All animals were sacrificed 24 hours after the last injection. Blood was collected by cardiac puncture into heparinized tubes, and the plasma used for RIA (radio immunoassay) of testosterone and luteinising hormone. The following organs were removed and weighed: adrenals, prostate, seminal vesicles, testes, kidneys. There was no significant body weight loss in any group of mice during the experiments.

Post mortem examination of the mice revealed oil/white deposits i.p. in those treated with compound of Ex. 1 and white deposits throughout the abdomen in those treated with compound of Ex. 4. In all these mice, all organs looked normal. In ketoconazole-treated animals, adhesions were found in 2/5,2/5,4/5 of the low/middle/top dose groups. The gut and peritoneal wall seemed to be stuck to the seminal vesicles. The livers were brown in the middle/top dose groups.

The weights of organs found in the animals *post mortem* are shown in Table 3 below. The reductions in weight of all of the porstate, seminal vesicles, testes and kidneys were much greater for the test compounds of the invention than for ketoconazole. Ketoconazole caused an increase in adrenal weight at the two highest doses, whereas the compounds of the invention had no significant effect, suggesting that they did not inhibit corticosterone biosynthesis.

TABLE 3

Compound of Ex 1.

Mean weight (mg.) ± standard error.

Dose	Adrenals	Prostate	Seminal Vesicles	Testes	Kidneys
controls	4.5 ± 0.1	10.1 ± 0.7	189 ± 9	146 ± 3	709 ± 17
solvent controls	4.5 ± 0.4	10.2 ± 1.3	171 ± 6	122 ± 7	615 ± 28
0.02mmol/ /kg/day	4.3 ± 0.2	8.0 ± 0.6	136 ± 4	134 ± 4	604 ± 24
0.1 mmol /kg/day	4.0 ± 0.2	5.3 ± 0.3	51 ± 6	95 ± 3	500 ± 8
0.5 mmol /kg/day	4.7 ± 0.2	5.6 ± 0.6	25 ± 2	56 ± 2	449 ± 12

Compound of Ex 4.

Dose	Adrenals	Prostate	Seminal Vesicles	Testes	Kidneys
controls	4.3 ± 0.4	8.4 ± 0.2	165 ± 18	142 ± 8	652 ± 45
solvent controls	4.4 ± 0.0	9.2 ± 0.9	152 ± 9	122 ± 8	589 ± 24
0.02mmol/ /kg/day	4.7 ± 0.2	5.9 ± 0.8	108 ± 4	117 ± 9	599 ± 29
0.1 mmol /kg/day	4.6 ± 0.4	6.4 ± 0.5	61 ± 9	105 ± 5	549 ± 28
0.5 mmol /kg/day	4.9 ± 0.1	4.1 ± 0.5	25 ± 1	59 ± 2	468 ± 15

Ketoconazole

Dose	Adrenals	Prostate	Seminal Vesicles	Testes	Kidneys
controls	4.2 ± 0.2	8.9 ± 0.8	193 ± 8	145 ± 4	670 ± 12
solvent controls	4.7 ± 0.4	9.3 ± 1.2	198 ± 18	146 ± 3	615 ± 25
0.01mmol/ /kg/day	4.8 ± 0.2	9.1 ± 0.8	235 ± 18	141 ± 5	637 ± 22
0.225 mmol /kg/day	6.1 ± 0.3	10.8 ± 1.4	171 ± 5	127 ± 7	574 ± 23
0.5 mmol /kg/day	6.9 ± 0.3	9.3 ± 0.9	179 ± 20	133 ± 6	710 ± 30

The results indicate the inhibition by the components of the invention of androgen and particularly testosterone synthesis. They are confirmed by endocrinological results shown in Table 4.

Although the solvent itself produced marked depression of testosterone levels, due to stress on the animals, the <u>further</u> decrease resulting from the administration of test compounds was much more marked for the compounds of the invention than for ketoconazole. The rise in LH levels is ascribed to a feedback mechanism associated with depletion of testosterone.

TABLE 4

Endocrinological Results (Mean ± se)

	Testosterone nM	LH ng/ml
Compound of Ex. 1		
Control	9.8 ± 5.6	0.63 ± 0.16
Solvent Control	2.5 ± 1.2	0.80 ± 0.09
0.02Mmol/Kg/Day	2.7 ± 0.5	3.4 ± 0.5
0.1Mmol/Kg/Day	0.2 ± 0.1	2.55 ± 0.45
0.5Mmol/Kg/Day	0.1 ± 0.0	2.25 ± 0.67
Compound of Ex. 4		
Control	27.8 ± 11.4	Not
solvent Control	11.0 ± 5.6	determined
0.02Mmol/Kg/Day	4.5 ± 0.3	
0.1Mmol/Kg/Day	3.5 ± 1.05	
0.5Mmol/Kg/Day	0.43 ± 0.14	

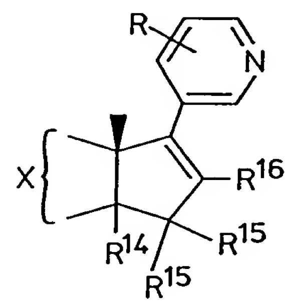
Ketoconazole

Control	17.3 ± 7.1	0.66 ± 0.05
Solvent Control	1.3 ± 0.4	0.25 ± 0.13
0.1Mmol/Kg/Day	0.9 ± 0.2	0.39 ± 0.14
0.225Mmol/Kg/Day	0.7 ± 0.15	0.75 ± 0.02
0.5Mmol/Kg/Day	0.4 ± 0.1	0.76 ± 0.03

The following claims define some important aspects of the invention, but do not purport to include every conceivable aspect for which protection might be sought in subsequent continuing and foreign patent applications, and should not be construed as detracting from the generality of the inventive concepts herein before described.

CLAIMS

1. Compounds of the general formula (1)



(1)

wherein X represents the residue of the A, B and C rings of a steroid, R represents a hydrogen atom or an alkyl group of 1-4 carbon atoms, R^{14} represents a hydrogen atom, a halogen atom or an alkyl group of 1 to 4 carbon atomsand each of the R^{15} substituents independently represents a hydrogen atom or an alkyl or alkoxy group of 1-4 carbon atoms, a hydroxy group or an alkylcarbonyloxy group of 2 to 5 carbon atoms or together represent an oxo or methylene group or R^{14} and one of the R^{15} groups together represent a double bond and the other R^{15} group represents a hydrogen atom or an alkyl group of 1 to 4 carbon atoms, and R^{16} represents a hydrogen atom, halogen atom, or an alkyl group of 1 to 4 carbon atoms, in the form of the free bases or pharmaceutically acceptable acid addition salts, with the proviso that 17-(3-pyridyl)androsta-5,14,16-trien-3β-ol and 15β-acetoxy-17-(3-pyridyl)androsta-5,16-dien-3β-ol and their 3-acetates and 3β-methoxy-17-(3-pyridyl)androst-16-ene are claimed only for use in therapy.

2. Compounds according to Claim 1 wherein X represents the residue of

androstan-3α- or 3β-ol,
androst-5-en-3α- or 3β-ol,
androst-4-en-3-one,
androst-2-ene
androst-4-ene
androst-5-ene

androsta-5,7-dien-3α or 3β-ol,
androsta-1,4-dien-3-one
androsta-3,5-diene,
estra-1,3,5[10]-triene or
estra-1,3,5[10]-trien-3-ol,

each of which, where structurally permissible, can be further derivatised in one or more of the following ways:

- to form 3-esters
- to have one or more carbon to carbon ring double bonds in any of the 5,6-, 6,7-, 7,8-, 9,11- and 11,12-positions
- as 3-oximes
- as 3-methylenes
- as 3-carboxylates
- as 3-nitriles
- as 3-nitros
- as 3-desoxy derivatives
- to have one or more hydroxy, halo, C_{1-4}-alkyl, trifluoromethyl, C_{1-4}-alkoxy, C_{1-4}-alkanoyloxy, benzoyloxy, oxo, methylene or alkenyl substituents in the A, B or C-ring
- to be 19-nor.

3. Compounds according to Claim 1 or 2 which are saturated and unsubstituted at the 11- and 12- positions.

4. 17-(3-pyridyl)androsta-5,16-dien-3β-ol,
17-(3-pyridyl)androsta-3,5,16-triene,
17-(3-pyridyl)androsta-4,16-dien-3-one,
17-(3-pyridyl)estra-1,3,5[10],16-tetraen-3-ol,
17-(3-pyridyl)-5α-androst-16-en-3α-ol
and their acid addition salts and 3-esters.

5. Compounds according to claim 1, 2 or 3 wherein R represents a hydrogen atom.

6. 17-(3-pyridyl)-5α-androst-16-en-3-one,
17-(3-pyridyl)-androsta-4, 16-diene-3,11-dione,
17-(3-pyridyl)-androsta-3,5,16-trien-3-ol,
6α-and 6β-fluoro-17-(3-pyridyl)androsta-4,16-dien-3-one

17-(3-pyridyl)androsta-4,16-dien-3,6-dione,

17-[3-(6-methylpyridyl)]androsta-5,16 dien-3β-ol

3α-triflurome thyl-17-(3-pyridyl)androst-16-en-3β-ol

and their acid addition salts and 3-esters.

7. A pharmaceutical composition comprising a compound claimed in Claim 1, 2, 3 or 6 in association with a pharmaceutically acceptable carrier or diluent.

8. Compounds according to Claim 1, 2, 3 or 6, for use in the therapy of androgen-dependent disorders.

9. Compounds according to Claim 7 for use in treating prostatic cancer.

10. Compounds according to Claim 1, 2, 3 or 6, for use in the therapy of oestrogen-dependent disorders.

11. Compounds according to Claim 9 for use in treating breast cancer.

12. A pharmaceutical composition comprising a compound claimed in Claim 4 or 5 in association with a pharmaceutically acceptable carrier or diluent.

13. Compounds according to Claim 4 or 5, for use in the therapy of androgen-dependent disorders.

14. Compounds according to Claim 13 for use in treating prostatic cancer.

15. Compounds according to Claim 4 or 5, for use in the therapy of oestrogen-dependent disorders.

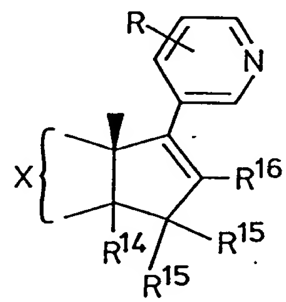
16. Compounds according to Claim 15 for use in treating breast cancer.

Figure (s) to accompany abstract

ABSTRACT

STEROIDS

Compounds of the general formula (1)



(1)

wherein X represents the residue of the A, B and C rings of a steroid, R represents a hydrogen atom or an alkyl group of 1 to 4 carbon atoms, R^{14} represents a hydrogen atom and R^{15} represents a hydrogen atom or an alkyl or alkoxy group of 1-4 carbon atoms, or a hydroxy or alkylcarbonyloxy group of 2 to 5 carbon atoms or R^{14} and R^{15} together represent a double bond, and R^{16} represents a hydrogen atom or an alkyl group of 1 to 4 carbon atoms, in the form of the free bases or pharmaceutically acceptable acid addition salts, with the proviso that 17-(3-pyridyl)androsta-5,14,16-trien-3β-ol and 15β-acetoxy-17-(3-pyridyl)androsta-5,16-dien-3β-ol and their 3-acetates and 3β-methoxy-17-(3-pyridyl)androst-16-ene are claimed only for use in therapy are useful for treatment of androgen-dependent disorders, especially prostatic cancer, and also oestrogen-dependent disorders such as breast cancer.

Ref: 92MAR/135279